Looking Across Data Streams to Draw Conclusions Regarding Causality: Key Considerations in the Formaldehyde Science

FORMALEHYDE SCIENCE INVITED EXPERTS WORKSHOP
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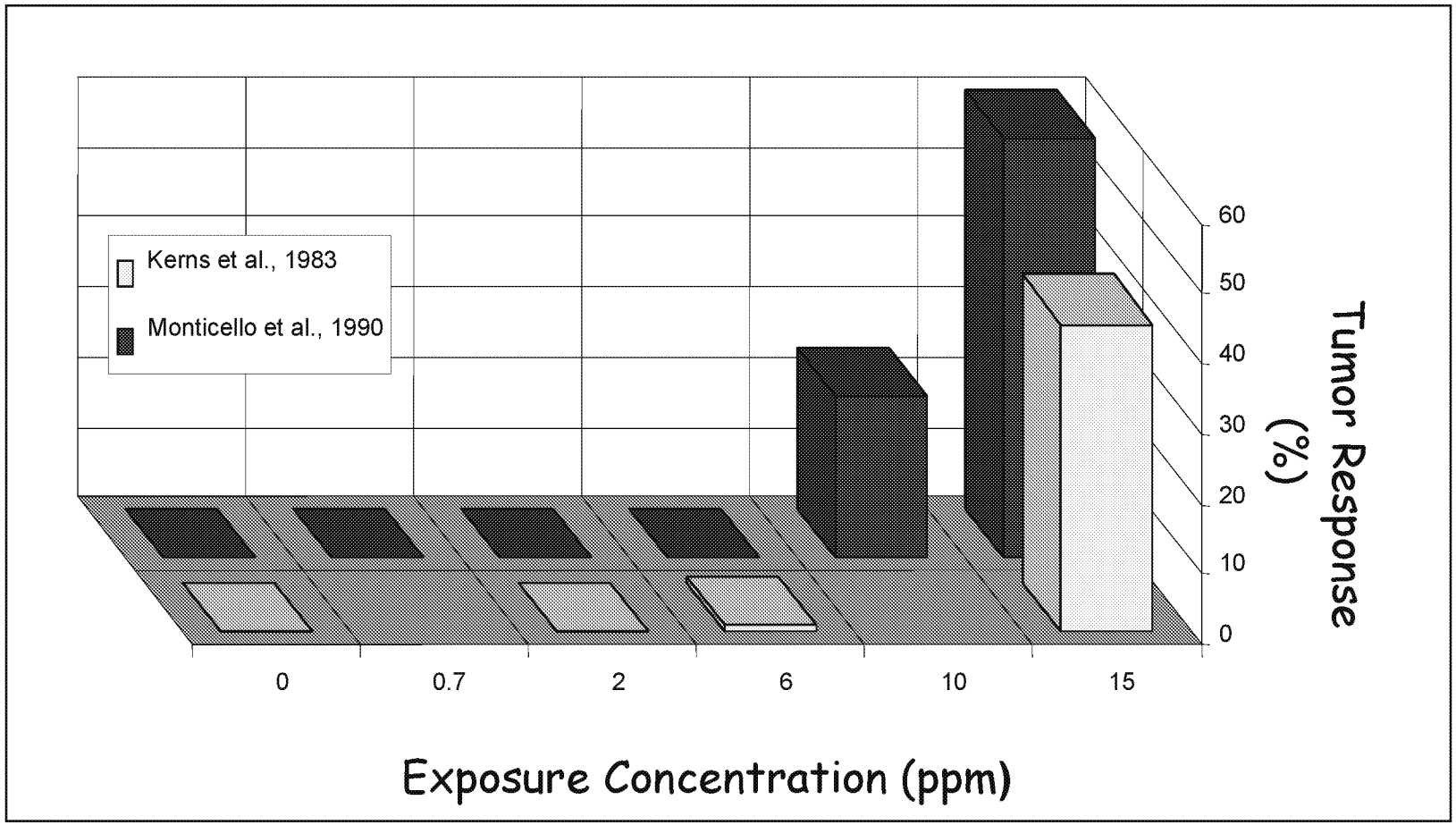
Potential Value of Different Data Streams

- Animal Bioassays:
  - hazard identification
  - dose-response (in experimental region)
- Epidemiology:
  - human relevance
  - dose-response
- Mechanistic studies:
  - mode of action (mutagenic, cytotoxic, receptor mediated)
  - low-dose extrapolation (linear, nonlinear)
- Modeling:
  - incorporation of mode of action in quantitative risk assessment
  - extrapolation (dose, routes, species)

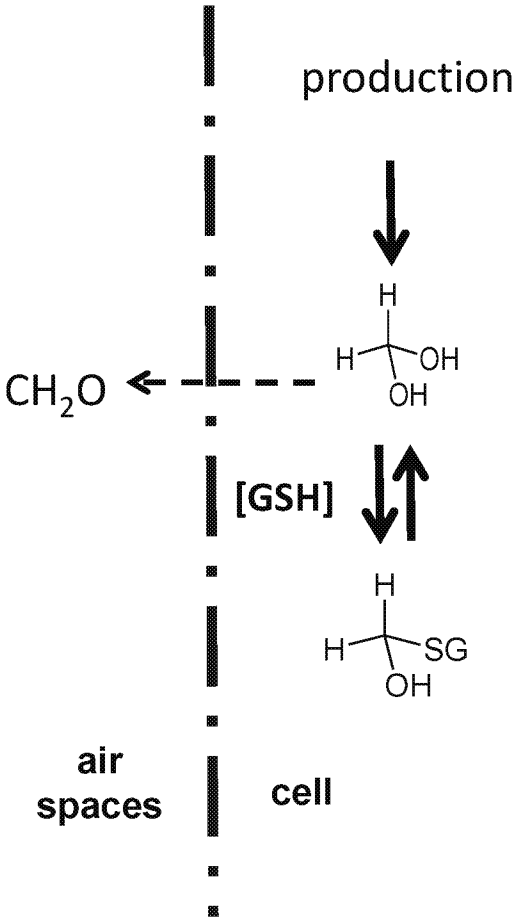
Uncertainties in Different Data Streams

- Animal Bioassays:
  - human relevance
  - dose-response at doses below experimental region
- Epidemiology:
  - impact of confounders, bias (Lash et al. 2012)
  - artificial linearization of dose-response (Crump 2005)
- Mechanistic studies:
  - how to incorporate in quantitative risk assessment
- Modeling:
  - only as good as the data its built on

Formaldehyde causes nasal tumors in rats…



...but it's a normal constituent of cells



- $CH_2(OH)_2$ is formed in cells by metabolism of amino acids and during one carbon pool metabolism.
- $CH_2(OH)_2$ complexes with glutathione to form hydroxymethylglutathione (K_{diss}~ 1.0 mM).
- Total tissue FA in the nasal mucosa in rats, in the absence of any inhalation exposure, was 0.42 $\pm$ 0.09 umoles/g (i.e., 12,600 ppb)

Cancer Risk Assessment Considerations for Formaldehyde

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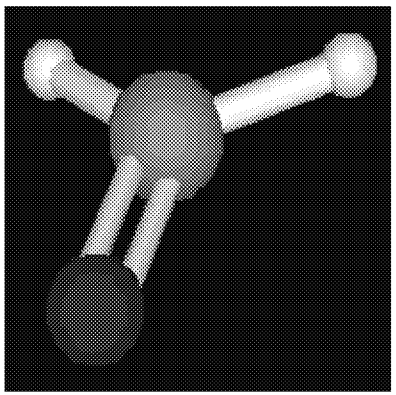


DNA interactions
- DNA-protein cross-links
- DNA mutation?

Increased cell turnover
- Due to toxicity

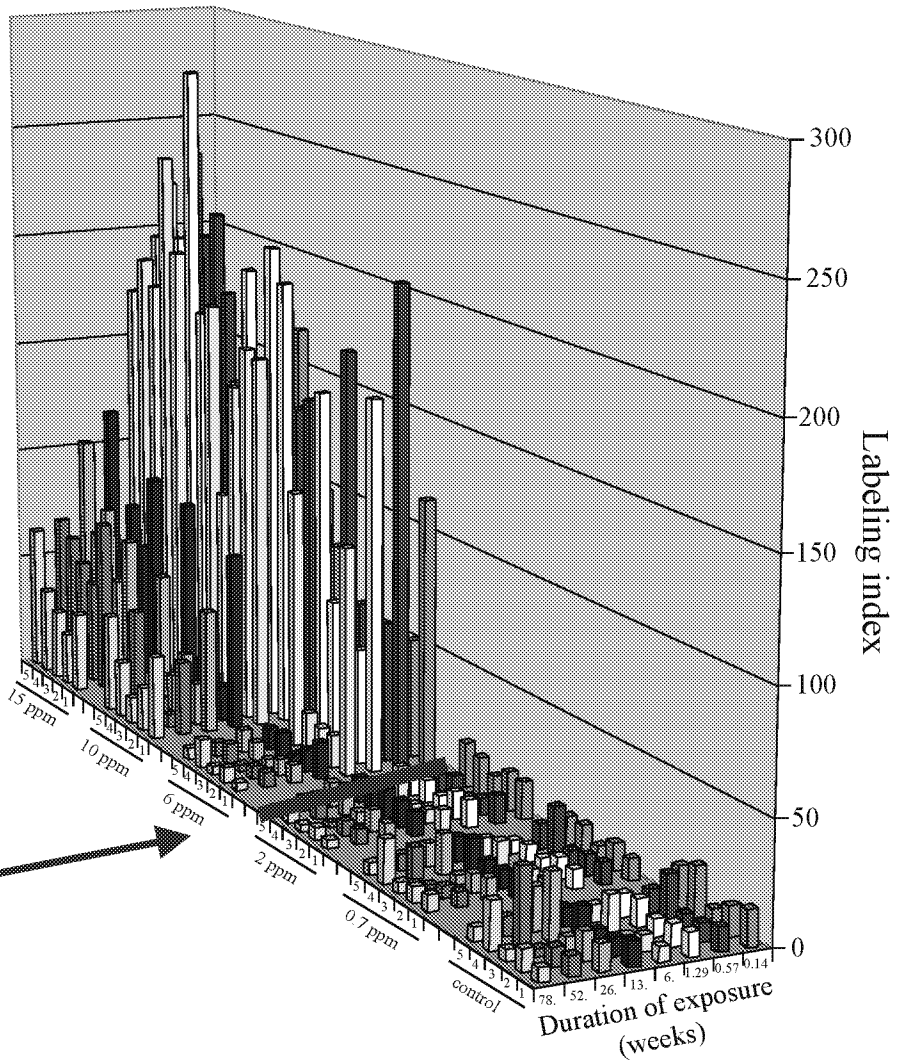
Tumor

Dosimetry *Modes of action* *Effects*

Cancer mode of action → expected dose/response

- Formaldehyde tumors occur at high inhaled concentrations (above 2 ppm) where severe toxicity leads to increased cell division to replace dead cells

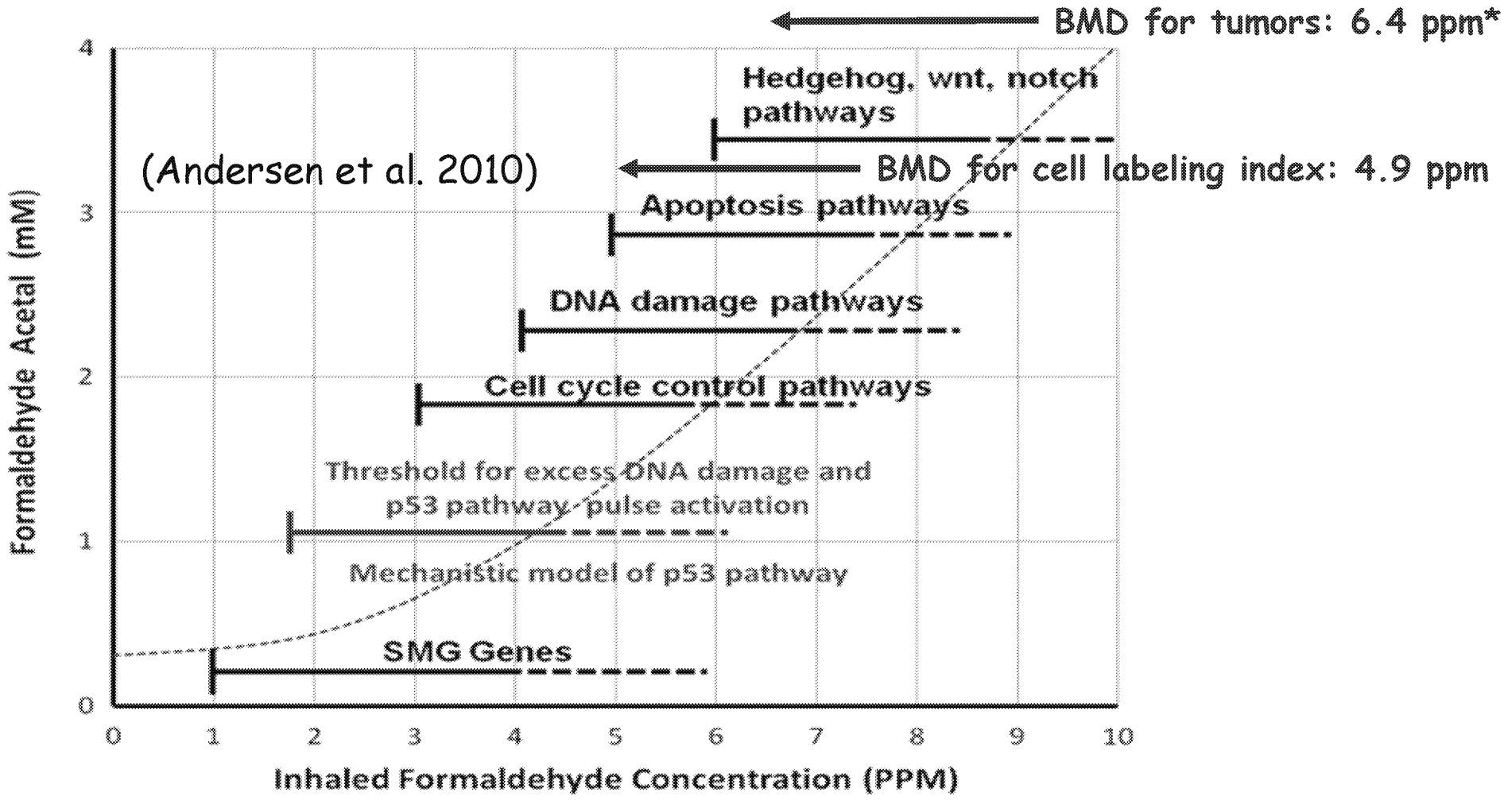
Formaldehyde is a potent irritant; the concentration that reduces breathing rate in mice by 50% (RD50) is 3 ppm



Genomic Markers Changed by Exposures

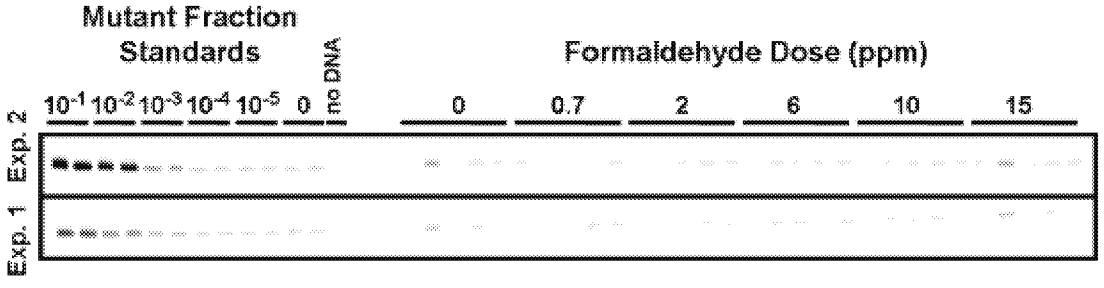
Sampling Time	0.7	2.0	6.0	15.0 ppm
6 hrs	**0**	**1**	**42**	**773**
1 day	**0**	**0**	**0**	
5 days	**0**	**15**	**28**	
8 days	**0**	**0**	**9**	
19 days	**0**	**0**	**48**	
13 weeks	**0**	**1**	**116**	**1394**

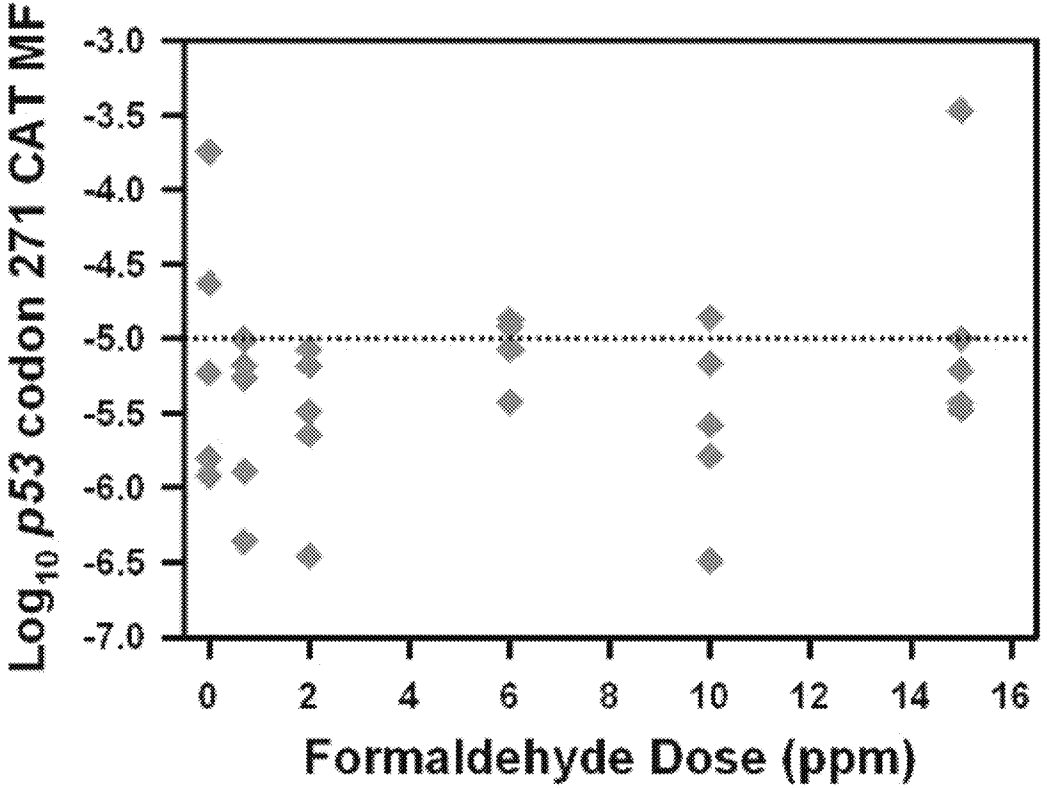
Comparison Between Genomic Dose Response and Tumor Response



*(Schlosser, Risk Anal., 2003)

Analysis of Mutation Frequency in p53 and K-ras Oncogenes





- NCTR analysis
- Found no increase in p53 or K-ras mutations after 90 days of exposure to formaldehyde at up to 15 ppm
- Demonstrates lack of mutagenic activity in vivo at carcinogenic concentrations

NAS Report on the EPA Risk Assessment for Formaldehyde

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Endogenous formaldehyde. Humans and other animals produce formaldehyde through various biologic pathways as part of normal metabolism. Thus, formaldehyde is normally present at low concentrations in all tissues, cells, and bodily fluids. Although there is some debate regarding interpretation of the analytic measurements, formaldehyde has been measured in exhaled breath and is most likely present normally at a concentration of a few parts per billion. The endogenous production of formaldehyde complicates the assessment of the risk associated with formaldehyde inhalation and remains an important uncertainty in assessing the additional dose received by inhalation, particularly at sites beyond the respiratory tract.

Usefulness of various models. Computational fluid dynamics (CFD) models have been developed to help to predict the dose to nasal tissues from inhaled formaldehyde. EPA fairly evaluated the models and sources of uncertainty but did not use the models to extrapolate to low concentrations. The committee concludes that the models would be useful for that purpose and recommends that EPA use the CFD models to extrapolate to low concentrations, include the results in the revised IRIS assessment, and explain clearly its use of CFD modeling approaches.

Given that the BBDR model for formaldehyde is one of the best-developed BBDR models to date, the positive attributes of BBDR models generally, and the limitations of the human data, the committee recommends that EPA use the BBDR model for formaldehyde in its cancer assessment, compare the results with those described in the draft assessment, and discuss the strengths and weaknesses of each approach.

The presence of endogenous formaldehyde has important low-dose risk assessment implications

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Formaldehyde Wall Mass Flux Predictions on Nonsquamous Epithelium in the Rat, Monkey, and Human Models

Exposure concentration (ppm)	Rat	Monkey	Human
	Maximum flux (pmol/(mm^2 h))		
1	9068.9	8570.2	10,183.8
0.1	898.6	870.3	1017.1
0.01	82.9	83.4	93.1
0.001	1.4	4.1	1.0×10^{-2}
	Average flux (pmol/(mm^2 h))		
1	503.0	1680.7	1551.2
0.1	49.8	169.4	148.8
0.01	4.6	15.7	13.5
0.001	0.1	0.8	-4.0×10^{-2}

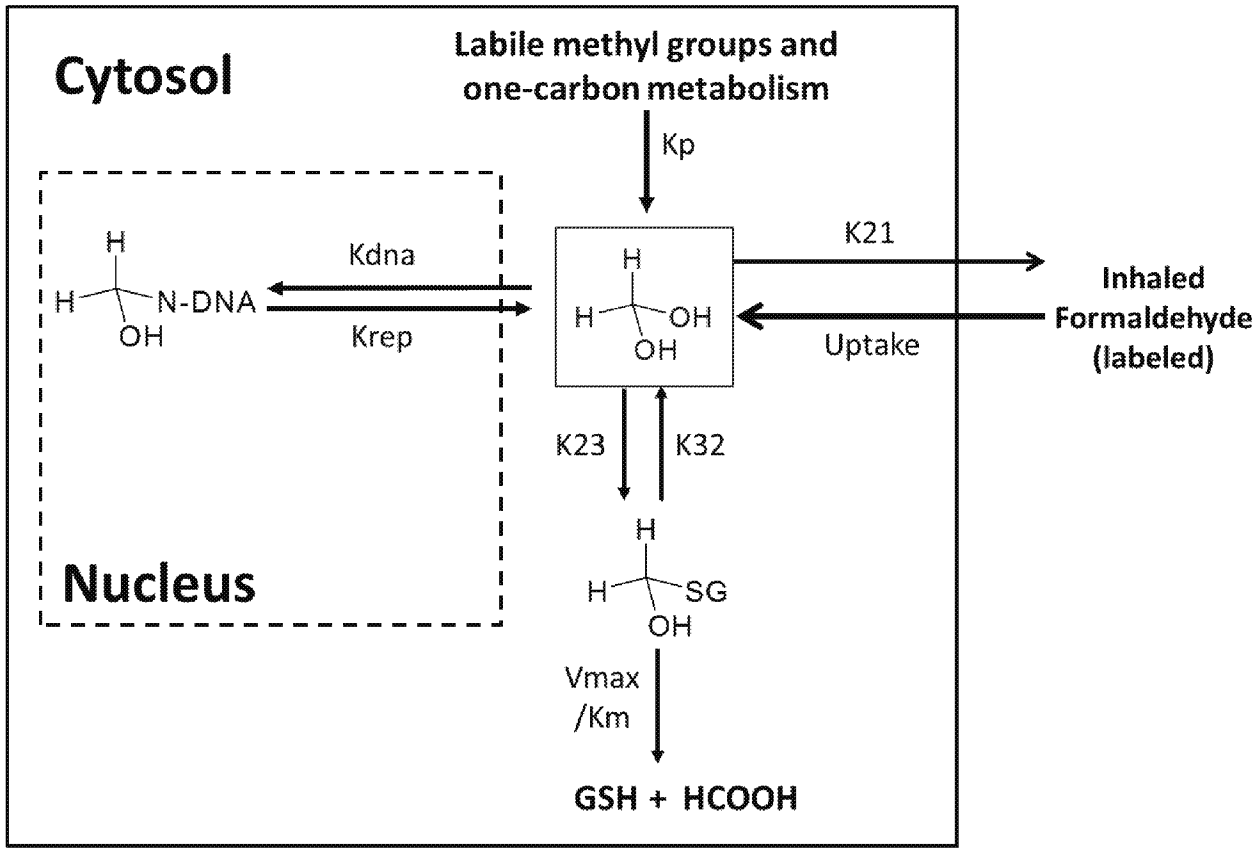
A simple approach for considering endogenous formaldehyde in a systemic cancer risk assessment (Swenberg et al. 2011)

TABLE 3

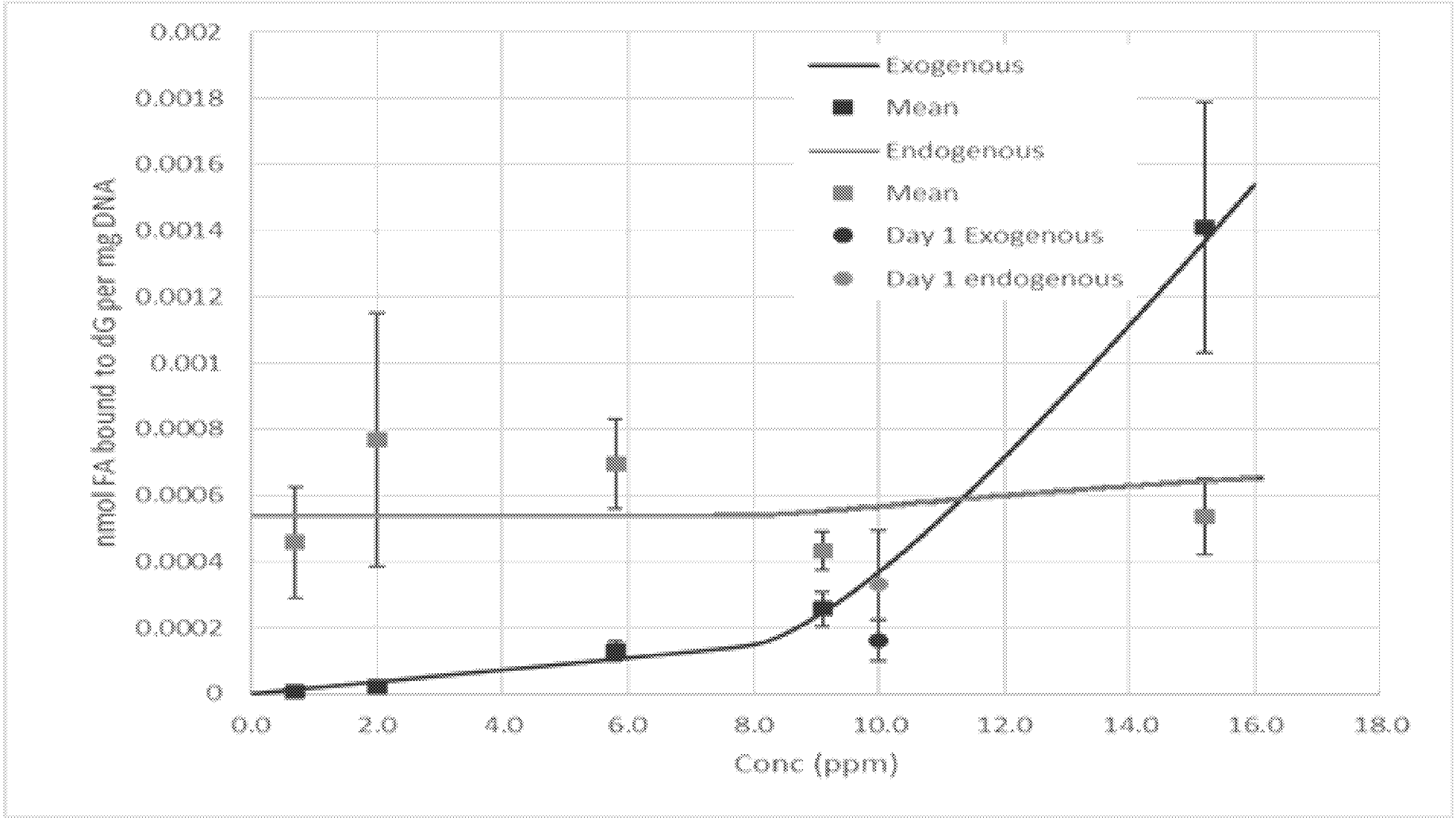
Comparison of Extra Lifetime Risks of Developing NPC, HL, and LEU from Continuous Exposure to 1 ppm Formaldehyde as Estimated Herein Using Formaldehyde-dG Adduct Data (dG-A) from Lu *et al.* (2010a), Lu *et al.* (2010b) and Moeller *et al.* (2011), with Those Estimated by U.S. EPA Using Adult Human Data (Table 6-3, pp. 6–41, 6–42, U.S. EPA draft document)

Cancer type	Background lifetime risk	Endogenous adducts, mean ± SE (per 10^7 dG)	UCL_{95} slope factor, risk per adduct (per 10^7 dG)	Exogenous adducts, mean ± SE (per 10^7 dG)	dG-A UCL_{95} risk estimate at 1 ppm	EPA UCL_{95} risk estimate at 1 ppm	Risk ratio, EPA/dG-A
NPC	7.25×10^{-4}	2.84 ± 0.51^a	3.61×10^{-4}	2.43 ± 0.29	1.22×10^{-3a}	1.1×10^{-2}	9.03
		2.63 ± 0.30^b	3.39×10^{-4}	1.28 ± 0.17	2.18×10^{-3b}		5.05
		4.24 ± 0.41^c	2.03×10^{-4}	11.15 ± 1.35	7.49×10^{-3c}		1.47
		3.41 ± 0.21^d	2.36×10^{-4}	2.03 ± 0.19	2.65×10^{-3d}		4.16
		5.51 ± 0.53^e	1.56×10^{-4}	1.04 ± 0.12	1.41×10^{-3e}		7.81
		6.09 ± 1.52^f	2.02×10^{-4}	0.19 ± 0.040	0.96×10^{-3f}		11.4
		3.62 ± 0.77^g	3.08×10^{-4}	0.039 ± 0.011	0.86×10^{-3g}		12.8
		2.05 ± 0.27^h	4.50×10^{-4}	0.41 ± 0.025	0.39×10^{-3h}		28.5
		2.49 ± 0.23^i	3.42×10^{-4}	0.25 ± 0.020	0.54×10^{-3i}		20.5
HL	2.3×10^{-3}	1.10 ± 0.16^a	2.76×10^{-3}	$< 1.77 \times 10^{-2j}$	$< 6.78 \times 10^{-5j}$	1.7×10^{-3}	> 251
		1.29 ± 0.19^b	2.36×10^{-3}	$< 1.77 \times 10^{-2j}$	$< 20.9 \times 10^{-5j}$		> 81.2
LEU	1.3×10^{-2}	1.17 ± 0.20^a	1.55×10^{-3}	$< 1.77 \times 10^{-2j}$	$< 3.81 \times 10^{-4j}$	5.7×10^{-2}	> 149.4
		1.05 ± 0.081^b	1.42×10^{-3}	$< 1.77 \times 10^{-2j}$	$< 12.6 \times 10^{-4j}$		> 45.2
		12.4 ± 1.82^h	1.38×10^{-3}	$< 1.03 \times 10^{-3k}$	$< 2.96 \times 10^{-6k}$		> 19,256
		17.5 ± 1.31^i	0.85×10^{-3}	$< 1.03 \times 10^{-3k}$	$< 5.47 \times 10^{-6k}$		> 10,420

A pharmacokinetic model that includes endogenous formaldehyde is now available for inclusion in the CIIT BBDR model

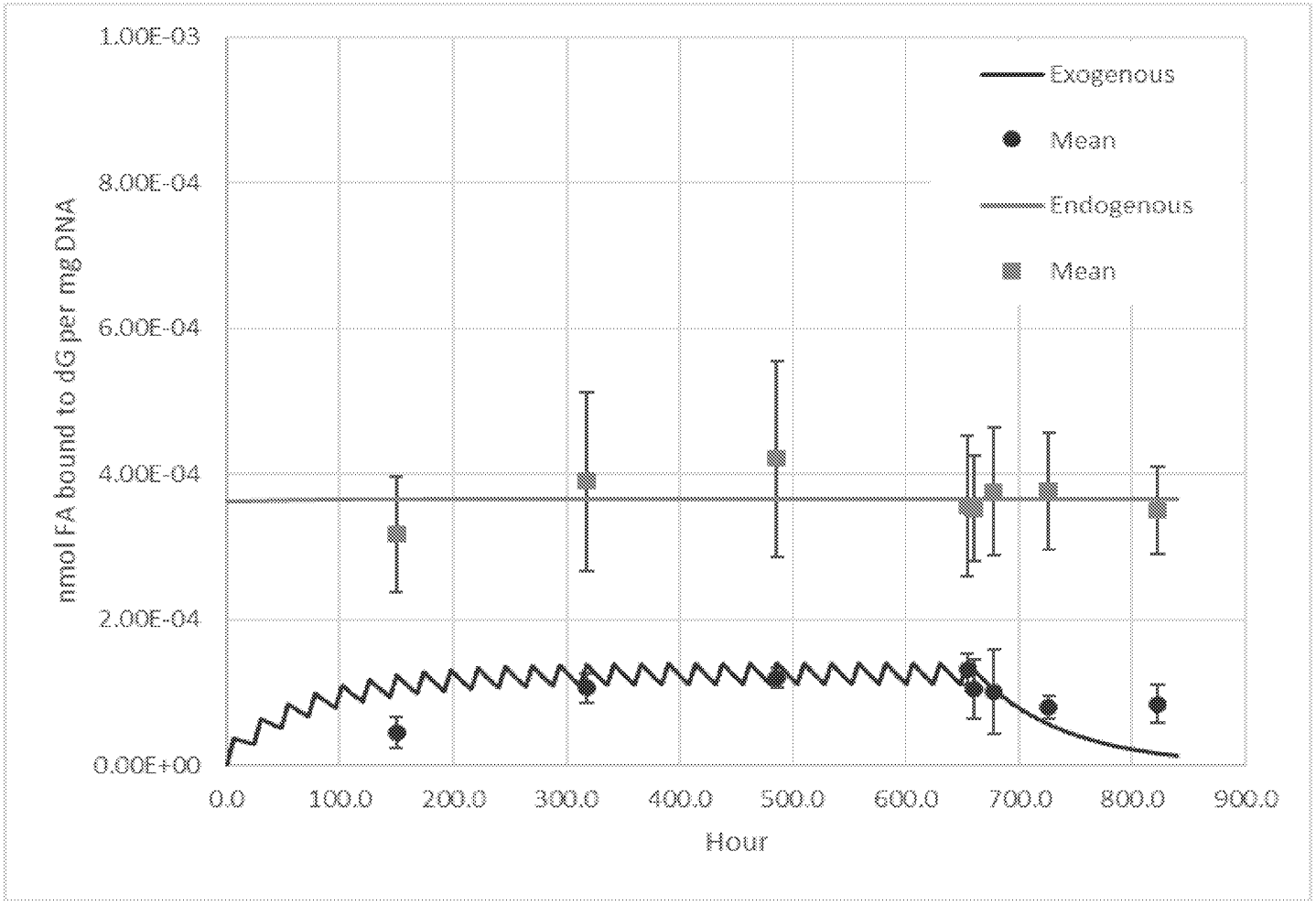


Endogenous and exogenous formaldehyde binding to dG in rat nasal tissue after a single 6-hour exposure to labelled formaldehyde concentrations of 1, 2, 6, 9, 10, and 15 ppm.



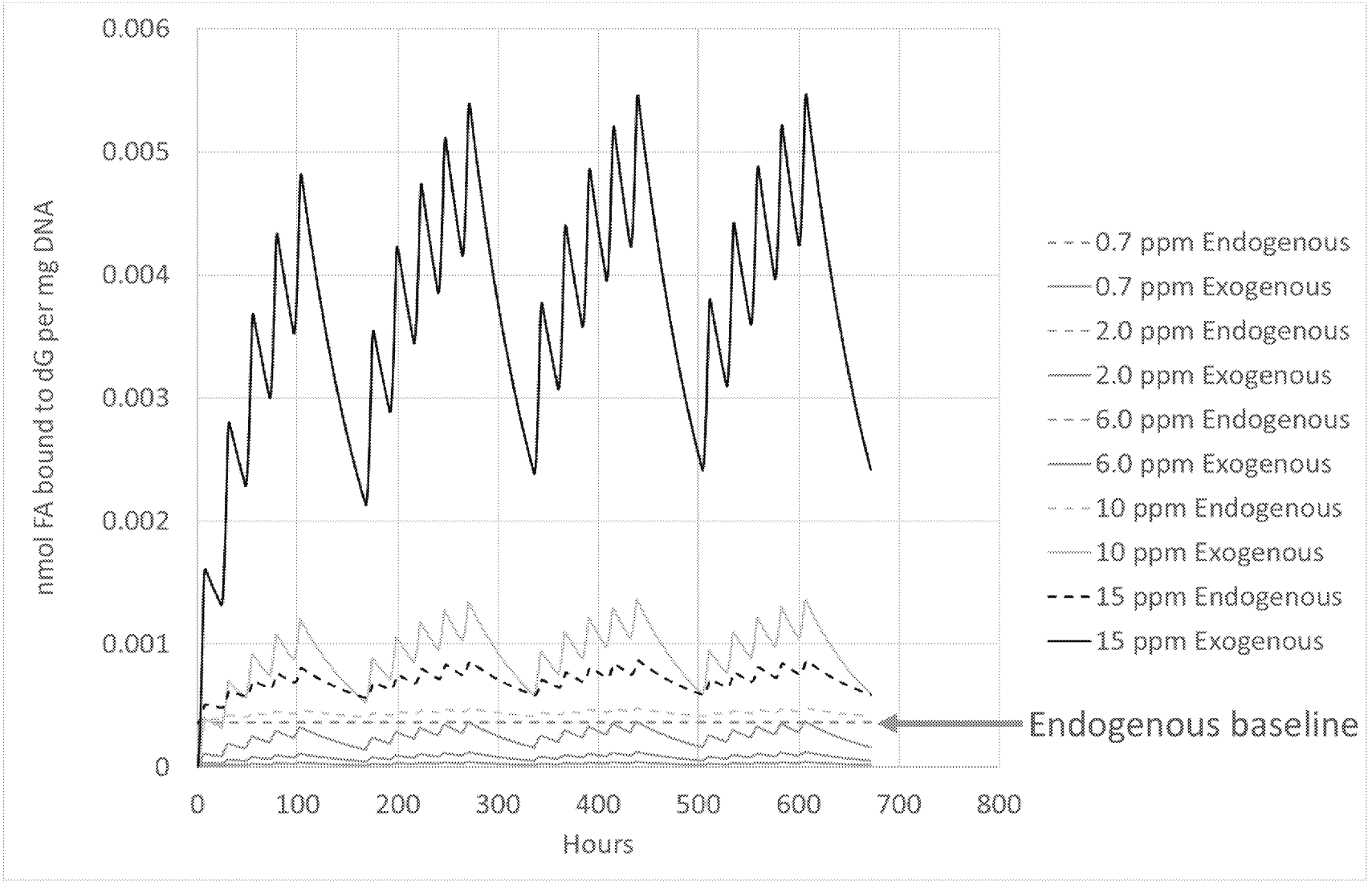
(circles: data from Lu et al. 2010; squares: data from Lu et al. 2011)

Endogenous (green) and exogenous (red) dG binding of formaldehyde in rat nasal tissue from exposure to 2 ppm formaldehyde 6 hours per day, 7 days per week, for 28 days.



(data from Yu et al. 2015)

Model-predicted endogenous (dashed lines) and exogenous (solid lines) formaldehyde-dG adducts at the exposure concentrations used in the 2-year inhalation bioassays in the rat (Kerns et al. 1983, Monticello et al. 1996)



Conclusions

- Genomic analysis shows that tumors occur at concentrations associated with severe cellular disruption (6 ppm and above)
- Mutation analysis shows no evidence of mutagenic activity at concentrations that are clearly toxic and tumorigenic
- Modeling of endogenous and exogenous DNA adduct data supports a nonlinear dose-dependence
- These mechanistic studies provide support for a threshold for formaldehyde carcinogenicity